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ARTÍCULO ORIGINAL

Influence of CYP3A4/5 polymorphisms in the pharmacokinetics of levonorgestrel: a pilot study

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Introduction. Levonorgestrel a synthetic progestagen used for endometriosis, dysmenorrhea and emergency contraception, is quickly and completely absorbed in the digestive tract. levonorgestrel is predominantly metabolised through hepatic routes that utilise the CYP3A system (CYP3A4 and CYP3A5).

Objective. This study aimed to evaluate the association between variant alleles of CYP3A4*1B and CYP3A5*3 polymorphisms and the pharmacokinetics of levonorgestrel.

Materials and methods. A group of 17 adult female healthy volunteers who signed an informed consent were genotyped for CYP3A4 and CYP3A5 through PCR-RFLP. Volunteers were submitted to pharmacokinetic analysis where, after a 12-hour overnight fast, they received a single oral dose of 0.75 mg of levonorgestrel. Serial blood samples were obtained (0 to 24 hours), and levonorgestrel concentrations were determined by UPLC-MS/MS to determine pharmacokinetic parameters. The procedures employed herein were performed according to the Declaration of Helsinki and Good Clinical Practices standards.

Results. Observed genotype frequencies in the studied group for CYP3A4*1B were 11.8% for *1B/*1B, 5.8% for *1/*1B and 82.4% for *1/*1. CYP3A5*3 frequencies were 70.5% for *3/*3, 23.5% for *1/*3 and 6.5% for *1/*1. A high pharmacokinetic variability between volunteers was observed, but no statistical association of pharmacokinetic parameters was found within the studied CYP3A4/5 polymorphisms.

Conclusions. Genetic polymorphisms could be important factors in determining inter-patient variability in plasma levonorgestrel concentrations, which in this study were not significantly associated with the presence of CYP3A4*1B and CYP3A5*3 polymorphisms. Therefore, due to the significant inter-patient variability that we observed during the course of this study, it is necessary to carry out studies with larger number of volunteers.

Key words: Levonorgestrel, hormones, pharmacogenetics.

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Influencia de polimorfismos genéticos de CYP3A4/5 en la farmacocinética de levonorgestrel: estudio piloto

Introducción. El levonorgestrel, un progestágeno sintético usado para endometriosis, dismenorrea y anticoncepción de emergencia, es rápida y completamente absorbido en el tubo digestivo. Su metabolismo es principalmente hepático, mediante las enzimas CYP3A4 y CYP3A5.

Objetivo. El presente estudio tuvo como objetivo evaluar la asociación entre la farmacocinética de levonorgestrel y las variantes alélicas de CYP3A4*1B y CYP3A5*3.

Materiales y métodos. En un grupo de 17 mujeres adultas sanas, que firmaron un consentimiento informado, se practicó genotipificación para CYP3A4*1B y CYP3A5*3 mediante PCR. Posteriormente, las voluntarias fueron sometidas a un estudio farmacocinético donde, luego de 12 horas de ayuno,

Author contributions:

Iván Saavedra, Luis Quiñones y Ángela Roco: diseño del estudio, supervisión de tesis de magíster de Iván Moreno y escritura del manuscrito.

Andrei Tchernitchin y Leonardo Gaete: obtención de financiamiento y revisión crítica del manuscrito.

Evelyn Tamayo y Jaime Sasso: apoyo analítico en cromatografía de masa, entrenamiento en dicha metodología a Iván Moreno y obtención de resultados iniciales.

Carla Miranda y Johanna Catalán: apoyo molecular en detección de variantes alélicas de CYP3A4 y CYP3A5, respectivamente. Entrenamiento a Iván Moreno en dichas metodologías moleculares. Obtención de primeros resultados.

recibieron una dosis de 0,75 mg de levonorgestrel. Se extrajeron muestras sanguíneas seriadas (0 a 24 horas) y se determinaron las concentraciones de levonorgestrel mediante un método validado de UPLC-ms/ms, para luego obtener los parámetros farmacocinéticos. Todos los procedimientos consideraron los aspectos éticos de la Declaración de Helsinki y las buenas prácticas clínicas.

Resultados. Las frecuencias genotípicas observadas para el grupo de estudio fueron 11,8 % para *1B/*1B; 5,8 % para *1/*1B, y 82,4 % para *1/*1 de CYP3A4*1B. Para CYP3A5*3, las frecuencias genotípicas fueron 70,5 % para *3/*3; 23,5 % para *1/*3, y 6,5 % para *1/*1. Se observa una interesante variabilidad entre las voluntarias que sugiere una relación con las variantes genéticas CYP3A, pero que no permite establecer una asociación estadísticamente significativa, presumiblemente debido al bajo número de individuos homocigotos mutados de CYP3A4 y silvestres de CYP3A5.

Conclusiones. Los polimorfismos genéticos podrían ser factores relevantes en la determinación de la variabilidad entre pacientes en las concentraciones plasmáticas de levonorgestrel, lo cual, sin embargo, no pudo ser establecido estadísticamente en este estudio. Por lo tanto, resulta necesario continuar este tipo de estudios con mayor número de voluntarios para establecer una asociación entre la variabilidad observada y la presencia de estos polimorfismos.

Palabras clave: levonorgestrel, hormonas, farmacogenética.

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Cytochrome P450 3A (CYP3A) enzymes constitute approximately 50% of the total hepatic amount of cytochrome P450 enzymes, and they are responsible for the metabolism of about half of the most commonly used drugs. This indicates that CYP3A enzymes present an unusually low substrate-specificity, which makes exposes them to reversible or irreversible inhibition by a wide variety of drugs (1-3). Due to this remarkable role on drug metabolism, it is important to know what parameters determine CYP3A activity. Moreover, there is evidence of significant inter-individual variability in the metabolic profile of CYP3A, which explains how the *in vivo* metabolism of CYP3A substrates may differ between individuals by up to a factor of ten in certain instances (4). CYP3A enzyme expression is induced by numerous drugs such as phenobarbital, rifampicin, dexamethasone and phenytoin. Known inhibitors of these enzymes include imidazole, antimycotics, antibiotics (such as macrolides) and some compounds in grapefruit juice (5).

Levonorgestrel, a synthetic progestagen derived from 19-nortestosterone, has been used for decades and is combined with ethynilestradiol as a contraceptive; this formulation has also been used for endometriosis, dysmenorrhea and emergency contraception (6-8). Levonorgestrel is quickly and completely absorbed in the digestive tract and is therefore is not affected by the first hepatic pass.

Thus, levonorgestrel has a bioavailability near 100%. After a single oral dose of 0.75 mg, the highest average plasmatic concentrations are 14 ng/mL after approximately 1.6 hours. (9). The active principle circulates in blood and is bound mainly to albumin and sexual hormone-binding globulin. The levonorgestrel half-life is approximately 20 to 30 hours. The active drug and its metabolites are excreted from the organism predominantly through the renal structure, though small amounts appear in faeces (10). Its metabolism is mainly hepatic, through the CYP3A system (CYP3A4 and CYP3A5). The CYP3A system is also responsible for the oxidative metabolism of endogenous steroids, such as oestrogens, progesterone and endogenous androgens (11-13).

There is not enough published data regarding the effects that CYP3A4*1B and CYP3A5*3 polymorphisms may have on the pharmacokinetic parameters of levonorgestrel in healthy volunteers. It is plausible that these polymorphisms may significantly inhibit levonorgestrel metabolism, which in turn raises the maximum plasmatic concentration (C_{max}), half-life ($t_{1/2}$), $AUC_{0 \rightarrow t}$, y $AUC_{0 \rightarrow \infty}$ and elimination constant (K_{el})

This study aimed to evaluate the association between variant alleles of CYP3A4*1B and CYP3A5*3 polymorphisms and the pharmacokinetics of levonorgestrel in a subgroup of healthy Chilean volunteers.

Materials and methods

Volunteers

A group of 17 adult female Chilean healthy volunteers, whose ages ranged from 18 to 50 years

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old, were chosen. Volunteers signed a written informed consent. Subsequently, a complete physical examination and clinical laboratory analysis were performed. This evaluation included hemogram, HSR (hematic sedimentation rate), urine analysis, HIV test, glycaemia, uraemia, proteinaemia, alkaline phosphatase, total bilirubin, ALT, AST and creatinine (table 1), in addition to a pregnancy test based on human chorionic gonadotropin hormone levels.

The inclusion criteria were as follows:

- Healthy Latin American women, between 18 and 50 years of age.
- Not pregnant or breast-feeding.
- Non-smokers, and neither drug users nor alcohol consumers.
- Without known drug allergies.
- Without concomitant therapies; neither hormonal nor psychotropic treatment and with

no history of consuming other drugs for at least three months prior to starting the study.

- Patients without any gynaecologic or malignant illness.
- With normal laboratory exams and declared suitable for the study by the presiding medical doctor.

Genotype analysis

Subjects were genotyped for CYP3A4 and CYP3A5 using the volunteer's peripheral blood samples and were isolated according to the DNA of the peripheral leukocytes, as defined by the laboratory's protocol. Polymerase Chain Reaction (PCR) was performed to study the polymorphisms in patients. For CYP3A4*1B, the method proposed by Cavalli, *et al.*, 2001 (14) was used, which uses the following *Forward* and *Reverse* primers, 5'-GGAATGAGGACAGCCATAGAGACAAGGGGA-3' and 5'-CCTTTCAGCTCTGTGTTGCTCTTTGCTG-3', respectively. These primers amplify the 5' promoter region of the human gene CYP3A4 (-318 to +6 nucleotides), producing a 385 bp length amplicon that contains the polymorphic (position 41) and constitutive (position 210) sites for the restriction enzyme *MbolI*. After performing the PCR protocol, the amplicon was digested by *MbolI* enzyme. Agarose 2.0% gel electrophoresis revealed 175 bp and 169 bp fragments of the wild type homozygous allele (*1/*1), 210 bp and 175 bp fragments for the homozygous polymorphic genotype (*1B/*1B), and fragments of 210, 175 and 169 bp for the heterozygous genotype (*1/*1B) (Figure 1A).

The polymorphism CYP3A5*3 was detected using the method proposed by Lee and Goldstein (15), which consists of an amplification with the *Forward* and *Reverse* primers, 5'-CTTTAAAGAGCTCTTTTGTCTCTCA-3' and 5'-

Table 1. Demographic characteristics and baseline data of hematological and biochemical parameters of 17 healthy adult female volunteers.

	Mean	SD	Normal range
Age (years)	26.94	8.76	-
Weight (kg)	59.56	7.23	-
Height (cm)	160.32	6.42	-
BMI	23.23	3.02	19-27
Creatinine (g/L)	0.83	0.19	0.8-1.5
Alkaline phosphatase (UI)	68.06	14.14	38.0-126.0
Glucose (mg/dl)	83.55	9.49	60.0-100.0
Uremia (mg/dl)	25.43	7.34	0.0-50.0
AST (UI)	24.47	6.57	5.0-40.0
ALT (UI)	17.71	6.95	7.0-56.0
Hematocrite	38.11	1.96	40.0-54.0
Leukocytes (x µl)	6620.0	1204.7	5,000.0-10,000.0
Hemoglobin (mg/dl)	12.73	0.73	12.0-16.0
Total bilirubin	0.57	0.26	0.2-1.3

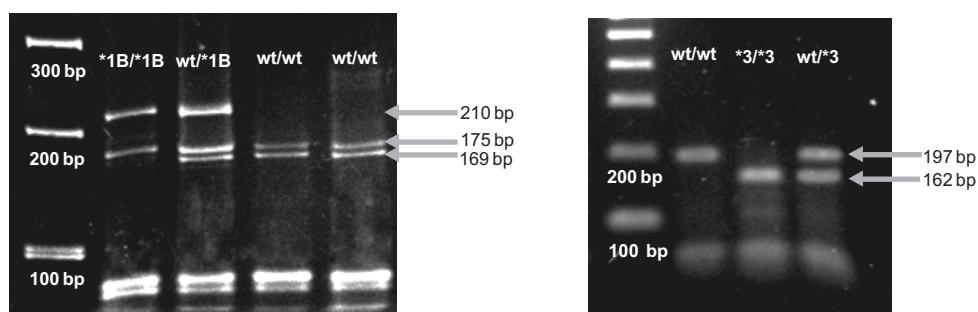


Figure 1. Agarose gel electrophoresis showing CYP3A4 (A) and CYP3A5 (B) genotypes

GAAGCCAGACTTTGATCATTATGTTATG-3', respectively. A non-matching nucleotide was used (underlined) on the forward primer, four bases upstream of the 3' end, to introduce an allele-specific restriction site for the *BseMII* enzyme. The protocol amplifies a fragment of intron 3 and produces a 197 bp long amplicon. Agarose 3% gel electrophoresis revealed the same 197 bp fragments for wild-type homozygous subjects (*1/*1), as well as without digestion, two fragments of 162 bp and 35 bp for subjects homozygous for polymorphism *3/*3, and three fragments of 197 bp, 162 bp and 35 bp for the heterozygous genotype (*1/*3) (Figure 1B).

Pharmacokinetic study

After a 12-hour overnight fast, volunteers received a single oral dose of 0.75 mg of levonorgestrel (Postinor™, Gedeon Richter Laboratories, Budapest, Hungary, Lot N° T69476) with 250 mL of drinking water while standing.

Blood samples were obtained in a heparinised glass tube before dosing (0) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12 and 24 hours post dose. After centrifugation, plasma samples were stored in plastic tubes at -20°C until assayed. Levonorgestrel concentrations were determined by Ultra Performance Liquid Chromatography (UPLC) connected with a mass spectrometer ACQUITY integrated system of Waters for levonorgestrel (16,17). The method was validated in the laboratory and defined according to sensibility, specificity, linearity, recovery, detection limits, quantification, accuracy, precision and reproducibility (both intraday and interday) for levonorgestrel concentration ranges of 0.5 to 90 ng/mL in 0.5 mL of plasma. The internal standard was 17- α -methyltestosterone (USP). Isolated plasma was defrosted and 1.0 mL was transferred to polypropylene tubes previously cleaned with methyl tert butyl ether (MTBE). Next, 5 mL of isoamyl hexane/alcohol (90/10) were added to extract the drug. The mixture was vortex-mixed for one minute and then centrifuged at 1400 g for 5 minutes. The sample was frozen, and the remaining liquid phase was evaporated by gaseous nitrogen, a process that takes between 15 to 20 minutes. Subsequently, the sample was reconstituted with the solvent used for the UPLC procedure (acetonitrile/water 69:31 with 0.1% formic acid). The mixture was then vortex-mixed. Chromatography was performed using a UPLC Waters system connected with a mass spectrometer, combined with a C18 (2.1 x 50 mm, 3 μ) Waters column. The mobile phases were acetonitrile/water 69:31 v/v with 0.1%

formic acid, injected at a rate of 200 μ L/min. The total elution time was 8.67 minutes.

The mass spectrometry was performed with a triple quadrupole mass spectrometry detector using electro spray ionisation for the mass analysis and detection. The mass spectrometric analysis was carried out in positive ion mode and arranged in the selective monitoring reaction mode. Based on all of the analytes' mass spectrums, the most abundant ions were selected, and the spectrometer was subsequently arranged to monitor the transitions of m/z 312.7 and 303.2 ions to the product ions m/z 108.96 and 96.88 for levonorgestrel and 17- α -methyltestosterone, respectively.

The following pharmacokinetic parameters were determined using the statistical computer's program Stata 10.0: maximum plasmatic concentration (C_{max}), maximum time (t_{max}), area under curve (AUC) of plasmatic concentrations from time zero to t_{max} and from 0 to infinity, half-life time ($t_{1/2}$) and elimination constant (K_e).

Ethics

The study protocols, both for the pharmacokinetic stage and the pharmacogenetic stage, were submitted separately to the Ethics Committee for Studies on Human Beings of the Faculty of Medicine, University of Chile, which approved both the protocols and the Informed Consent document provided by the volunteers. Procedures employed were in accordance with the declaration of Helsinki and Good Clinical Practices of the FDA (18,19).

Statistical analysis

Statistical analysis was carried out with statistical program Stata 10. To relate pharmacokinetic data to the different studied genotypes, the Kruskal-Wallis test was applied; this test applied a unilateral ANOVA of variation ranges, to each studied polymorphism, using a significance value of 0.05.

Results

All subjects successfully completed the study in accordance with the protocol. The genotype analysis of healthy volunteers unveiled the following results: for CYP3A4*1B, 14 were wild type (*1), 1 heterozygous (*1/*1B) and 2 homozygous for the polymorphism (*1B/*1B); for CYP3A5*3 there was 1 wild-type (*1/*1), 4 heterozygous (*1/*3) and 12 homozygous for the polymorphism (*3/*3).

Demographic data showed no significant differences according to CYP3A4 and CYP3A5 genotype

(data no shown). The studied group had a 29.6% Amerindian-Caucasian admixture, determined using the method of Acuña, *et al.*, 2000 (20).

The pharmacokinetic data of the volunteers according to their genotypes for CYP3A4 and CYP3A5 are shown on table 2.

Genotype frequencies for CYP3A4*1B were 11.8% for *1B/*1B, 5.8 % for *1/*1B and 82.4% for *1/*1. The allele frequency for *1B allele was 0.147. For CYP3A5*3 polymorphism genotype frequencies were 70.5 % for *3/*3, 23.5 % for *1/*3 and 6.5% for *1/*1. The allele frequency for *3 was 0.824.

Pharmacokinetic parameters separated by genotype are shown also in table 2 and figure 2. The statistical analysis show no significant differences in C_{max} among volunteers with *1/*1, *1B/*1B and *1/*1B, or among any of the CYP3A5 genotypes.

Discussion

CYP3A4*1B consists of a SNP present in the gene's promoter region (-292) which seems to produce altered enzymatic activity. *In vitro*, it exhibits twice the activity compared to the wild-type genotype CYP3A4*1A (21), even though there are no *in vivo* studies showing that the CYP3A4*1B polymorphism is linked to a higher enzyme activity (22). Recently, Schirmer *et al.*, 2007 (23) proposed a role for this polymorphism and other CYP3A variants on the catalytic activity of the enzyme. The authors try to reconcile the apparent contradiction between the evidence for the influence of the individual genetic makeup on CYP3A4 expression and activity suggested by clinical studies, and the failure to identify the responsible CYP3A gene variants. However, the studied CYP3A5 polymorphism

(CYP3A5*3) is found in the intron 3 (6986 A>G) and produces a splice variant that originates in the aberrant exon 3B, which causes the introduction of a premature stop codon and leads to the translation of a non-functional truncated protein (24). This enzyme possesses a large structural and catalytic resemblance with CYP3A4, and even though it is unlikely that this enzyme is expressed in a polymorphic way in tissues, its contribution to the clearance of some drugs may be an important source of inter-individual variability (1,24,25). There are no reported studies regarding the role of the polymorphism CYP3A5*3 and levonorgestrel metabolism.

The allelic frequencies found in the healthy Chilean volunteers are similar to those reported within the Caucasian and Japanese populations (1,22,26), showing no relevant differences in our "mestizo" population in terms of the presence of the studied variants. This observation stands in opposition to other polymorphism for phase I and phase II biotransformation enzymes, in which we have been able to observe profound differences (26).

Because levonorgestrel is a substrate of the studied cytochromes (CYP3A4 and CYP3A5), we proposed that the studied polymorphism may have an effect on levonorgestrel pharmacokinetic parameters in healthy volunteers treated with a single, oral dose of the drug. Moreover, as it is well known these parameters also can be influenced by other factors such as physiological and pathological conditions, environmental exposure and interactions with other drugs, we strictly controlled health conditions. We ensured the volunteers did not consume any medication and

Table 2. Pharmacokinetic data according to the CYP3A4 and CYP3A5 genotypes of 17 adult female healthy volunteers

	CYP3A4*1/*1	CYP3A4*1/*1B	CYP3A4*1B/*1B
Number of subjects	14	1	2
C_{max} (ng/ml)	16.35± 9.5	23.97	16.98± 1.6
$T_{1/2}$ (hours)	23.91± 13.5	53,13	21.41± 5,8
$AUC_{0 \rightarrow T_{max}}$ (ng.h/ml)	110.13± 35.7	225.85	115.31± 33.0
$AUC_{0 \rightarrow \infty}$ (ng.h/ml)	191.95± 16.1	724.58	205.47± 92.3
Ke (1/time in hours)	0.032± 0.016	0.010	0.04± 0.005
	CYP3A5*1/*1	CYP3A5*1/*3	CYP3A5*3/3
Number of subjects	1	4	12
C_{max} : ng/ml (SD)	18.51	22.90 (13.9)	14.20 (5.9)
$T_{1/2}$: hours (SD)	27.29	16.62 (4.6)	28.07 (15.7)
$AUC_{0 \rightarrow T_{max}}$: ng.h/ml (SD)	148.34	204.08 (127.5)	86.13 (54.23)
$AUC_{0 \rightarrow \infty}$: ng.h/ml (SD)	297.80	309.59 (214.5)	190.56 (32.3)
Ke: 1/time in hours (SD)	0.02	0.04 (0.01)	0.027 (0.014)

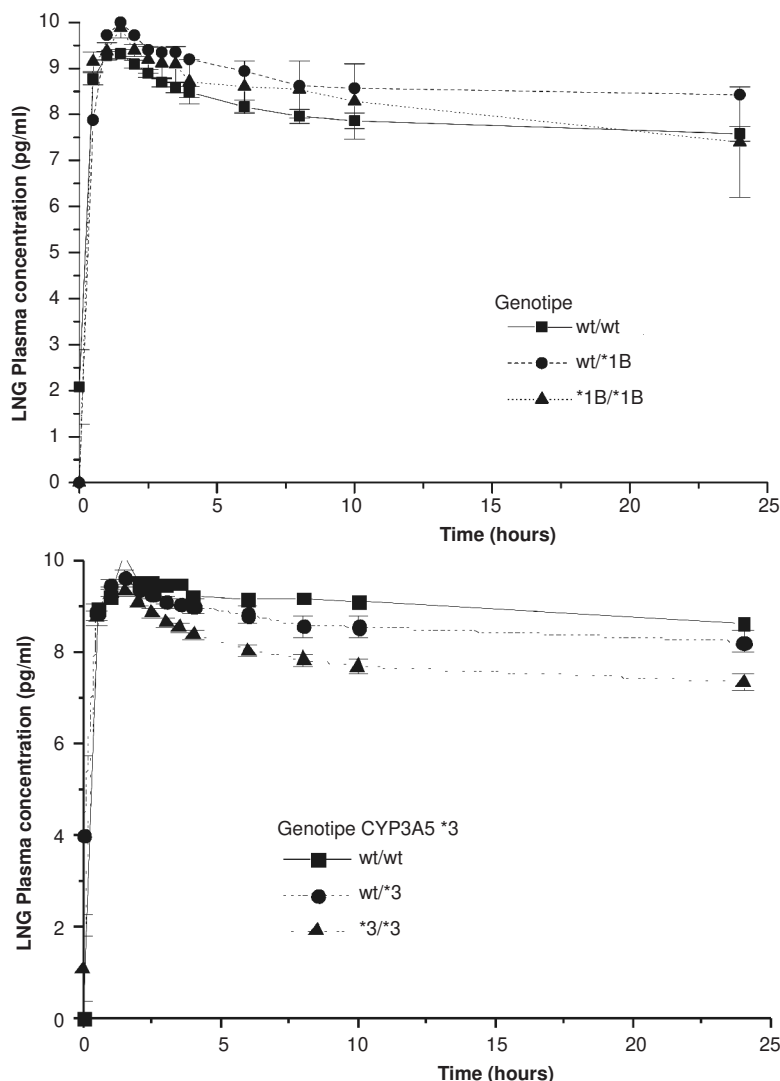


Figure 2. Pharmacokinetic curves of volunteers based on CYP3A4*1B (A) and CYP3A5*3 (B) genotypes

that they were not exposed to any environmental or alimentary relevant xenobiotics.

We used a validated UPLC MS/MS method to detect levonorgestrel similar to that usually employed in the detection of other progestagens and oestrogens. This method provides high instrument sensibility and, because we are looking for the parent drug and not the metabolite, we can observe LNG plasma levels to evaluate the bioavailability as an indication of the potential therapeutic effect.

The results showed no significant differences among the pharmacokinetic parameters analysed (C_{max} , $t_{1/2}$, $AUC_{0 \rightarrow t_{max}}$, $AUC_{0 \rightarrow \infty}$ and Ke) for CYP3A4*1B and CYP3A5*3 polymorphisms in the studied group. This fact could be due to the small number of subjects in the current study.

To the best of our knowledge, this is a first study to look for a relationship between CYP3A4/5 polymorphisms and pharmacokinetics of levonorgestrel. Thus, it could be a first approach to the personalised progestagen treatment based on pharmacogenetic profiles.

Despite the fact we were not able to demonstrate significant differences, the observation that volunteers have heterogeneous pharmacokinetic parameters in their individual pharmacokinetic curves (data not shown); additionally, the observations, based on the genotype-grouped curves (figure 2), provides supplemental tools for studying possible personalised pharmacotherapy. Undoubtedly, it is necessary to continue these investigations by carrying out studies with a larger number of volunteers.

In combination with other studies, our study could help us to define the potential clinical use for therapeutics in populations containing genetic polymorphisms in CYP3A4 and CYP3A5 genes, such as the Amerindian-Caucasian admixtures observed in the Chilean population. Therefore, the data obtained might assist in our understanding of the inter-ethnic differences for not only single CYP3A polymorphisms but also the function of simultaneous CYP3A4/5 polymorphisms, and they may help explain the differences in the metabolic responses to levonorgestrel among different individuals.

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Conflict of interests

The authors declare that they have no conflicts of interest.

References

1. **Guengerich FP.** Human Cytochrome P450 enzymes. In: Ortiz PR, editor. *Cytochrome P450: Structure, mechanism, and biochemistry*. Third edition. New York: Kluwer Academic/Plenum Publishers; 2005. p. 377-530.
2. **Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP.** Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther*. 1994;270:414-23.
3. **Thummel KE, Wilkinson GR.** *In vitro* and *in vivo* drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol*. 1998;38:389-430. <http://dx.doi.org/10.1146/annurev.pharmtox.38.1.389>
4. **Guengerich FP.** Cytochrome P450 3A4: Regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol*. 1999;39:1-17. <http://dx.doi.org/10.1146/annurev.pharmtox.39.1.1>
5. **Frye R, Fitzgerald S, Lagattuta T, Hruska M y Egorin M.** Effect of St. John's work on imatinib mesylate pharmacokinetics. *Clin Pharmacol Ther*. 2004;76:323-9. <http://dx.doi.org/10.1016/j.clpt.2004.06.007>
6. **Brunton LL, Lazo JS, Parker KL.** Estrogens and progestins. En: Loose DS, Stancel GM, editors. *Goodman & Gilman's The pharmacological basis of therapeutics*. 11th edition. New York: McGraw-Hill; 2006. p. 1541-71.
7. **Langston A.** Emergency contraception: Update and review. *Semin Reprod Med*. 2010;28:95-102. <http://dx.doi.org/10.1055/s-0030-1248133>
8. **Wagstaff AJ.** Continuous-use ethinylestradiol/levonorgestrel 20microg/90microg: As an oral contraceptive. *Drugs*. 2007;67:2473-7.
9. **Paine MJ, Scrutton NS, Munro AW, Gutiérrez A, Roberts GC, Wolf CR.** Electron transfer partners of cytochrome P450. In: Ortiz PR, editor. *Cytochrome P450: Structure, mechanism, and biochemistry*. Third edition. New York: Kluwer Academic/Plenum Publishers; 2005. p. 115-48.
10. **Yamazaki H, Tsutomu S.** Progesterone and testosterone hydroxylation by cytochromes P450 2C19, 2C9 and 3A4 in human liver microsomes. *Arch Biochem Biophys*. 1997;346:161-9. <http://dx.doi.org/10.1006/abbi.1997.0302>
11. **Stanczyk FZ, Roy C.** Metabolism of levonorgestrel, norethindrone and structurally related contraceptive steroids. *Contraception*. 1990;42:67-96. [http://dx.doi.org/10.1016/0010-7824\(90\)90093-B](http://dx.doi.org/10.1016/0010-7824(90)90093-B)
12. **Suk Wai N, Fan S, Li S, Linan Ch, Ding J, Xiaoping J, et al.** A randomized trial to compare 24 h versus 12 h double dose regimen of levonorgestrel for emergency contraception. *Hum Reprod*. 2005;20:307-11. <http://dx.doi.org/10.1093/humrep/deh583>
13. **Zhao LZ, Zhong GP, Bi H, Ding L, Deng Y, Guan S, et al.** Determination of levonorgestrel in human plasma by liquid chromatography-tandem mass spectrometry method: Application to a bioequivalence study of two formulations in healthy volunteers. *Biomed Chromatogr*. 2008;22:519-26. <http://dx.doi.org/10.1002/bmc.963>
14. **Cavalli SA, Hirata MH, Hirata RD.** Detection of *MbolI* polymorphism at the 5' promoter region of CYP3A4. *Clin Chem*. 2001;47:348-51.
15. **Lee SJ, Goldstein JA.** Functionally defective or altered CYP3A4 and CYP3A5 single nucleotide polymorphisms and their detection with genotyping tests. *Pharmacogenomics*. 2005;6:357-71. <http://dx.doi.org/10.1517/14622416.6.4.357>
16. **Theron HB, Coetzee C, Sutherland F, Wiesner J, Swart K.** Selective and sensitive liquid chromatography-tandem mass spectrometry method for the determination of levonorgestrel in human plasma. *J Chromatogr B Anal Technol Biomed Life Sci*. 2004;813:331-6. <http://dx.doi.org/10.1016/j.jchromb.2004.10.039>
17. **Sambola NC, Harper C, Kim L, Liua Ch, Darney P, Raine T.** Pharmacokinetics of single-dose levonorgestrel in adolescents. *Contraception*. 2006;74:104-9. <http://dx.doi.org/10.1016/j.contraception.2006.01.011>
18. **Nuremberg Doctors' Trial.** Declaration of Helsinki (1964). *BMJ*. 1996;313:1448-9. <http://dx.doi.org/10.1136/bmj.313.7070.1448a>
19. **Food and Drug Administration.** Guidance for Industry: E6 Good Clinical Practice: Consolidate guidance. Rockville, MD: U.S. Dept. of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Control, Center for Biologics Evaluation and Research; 1996.
20. **Acuña M, Llop E, Rothhammer F.** Composición genética de la población chilena: las comunidades rurales de los valles del Elqui, Limarí y Choapa. *Rev Med Chile*. 2000;128:593-600. <http://dx.doi.org/10.4067/S0034-98872000000600004>
21. **Amirami B, Weber B, Rebbeck T.** Regulation of reporter gene expression by a CYP3A4 promoter variant in primary human hepatocytes. *Proc Am Assoc Cancer Res*. 2000;60:114.
22. **Wandel C, Witte J, Hall J, Stein C, Wood A, Wilkinson G.** CYP3A4 activity in African American and European American men: population differences in hepatic expression of CYP3A4, relationship to genetic polymorphism in the 5'-

- upstream regulatory region. Clin Pharmacol Ther. 2000; 68:82-91.
23. **Schirmer M, Rosenberger A, Klein K, Kulle B, Toliat M, Nürnberg P, et al.** Sex-dependent genetic markers of CYP3A4 expression and activity in human liver microsomes. Pharmacogenomics. 2007;8:443-53. <http://dx.doi.org/10.2217/14622416.8.5.443>
24. **Kuehl P, Zhang J, Lin Y, Brockmoller J, Hustert E.** Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet. 2001;27:383-91. <http://dx.doi.org/10.1038/86882>
25. **Huang W, Lin Y, McConn II D, Calamia J, Totah R, Isoherranen N, et al.** Evidence of significant contribution from CYP3A5 to hepatic drug metabolism. Drug Metab Dispos. 2004;32:1434-45. <http://dx.doi.org/10.1124/dmd.104.001313>
26. **Roco A, Quiñones L, Agúndez JA, García-Martín E, Squicciarini V, Miranda C, et al.** frequencies of 23 Functionally significant variant alleles related with metabolism of antineoplastic drugs in the Chilean population: comparison with Caucasian and Asian populations. Front Gene. 2010;3:229. <http://dx.doi.org/10.3389/fgene.2012.00229>